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Algal Nutrient Studies of the Potomac Estuary

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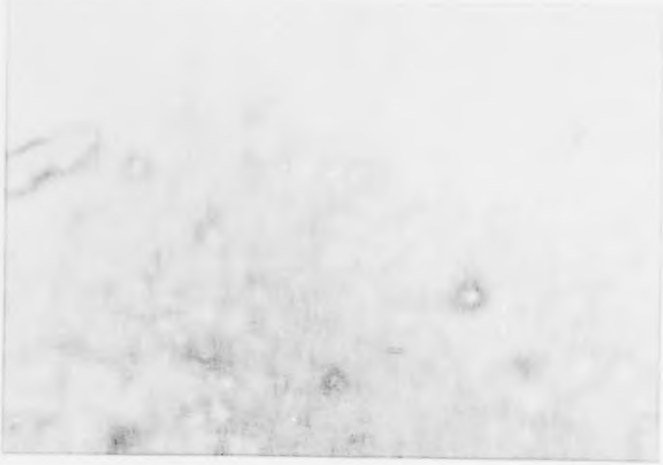

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16. ABSTRACT

The nutrient requirements of the phytoplankton of the Potomac Estuary were studied during the summer of 1977 employing the following laboratory tests: NH_4^+-N uptake, alkaline phosphatase enzyme activity; extractable surplus orthophosphate; tissue analysis for carbon, nitrogen and phosphorus content; and nitrogen fixation by acetylene reduction. The results indicated that the bloom of Oscillatoria was limited by nitrogen and that adequate phosphorus was present.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Algae Nutrients	Luxury phosphorus Ammonium uptake Nitrogen fixation Alkaline phosphatase Elemental analysis	

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ALGAL NUTRIENT STUDIES OF THE POTOMAC ESTUARY

(Summer 1977)

Annapolis Field Office
Region III
Environmental Protection Agency

Joseph Lee Slayton
E. R. Trovato

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I. Introduction

During the summer of 1977 an intensive survey of the middle reach of the Potomac River (Figure 1, Table 1) was undertaken by the A.F.O. As part of this work the nutrient requirements of the phytoplankton present were studied using the following laboratory tests: NH_4^+-N uptake; alkaline phosphatase enzyme activity; extractable surplus orthophoshate; tissue analysis for carbon, nitrogen and phosphorus content; and nitrogen fixation by acetylene reduction. These bio-assays were conducted in the Potomac from Gunston Cove to Possum Point during August and September 1977.

The ammonium uptake test was designed to assess the bio-availability of nitrogen to algae. Algae are spiked with ammonia and if a rapid rate of absorption of nitrogen with time is observed this signifies that nitrogen is limiting potential algal growth.

Algae have the ability to store phosphorus[1] when it is encountered in amounts beyond the immediate biological need. Previous studies[2] have determined that this stored phosphorus is easily extracted and is thought to be stored as orthophosphate; polyphosphate chains and/or as very labile organic compounds which breakdown to orthophosphate with heat (100°C). Algae containing significant luxury phosphate are not limited in their growth by phosphorus.

When ambient bio-available phosphorus is depleted in the water column, algae may activate the production of alkaline phosphatase enzyme. This enzyme cleaves phosphate from the stored luxury phosphate chains/compounds. The presence of significant alkaline phosphate enzyme is indicative of algae limited in their potential growth by phosphorus

Figure 1. Study Area

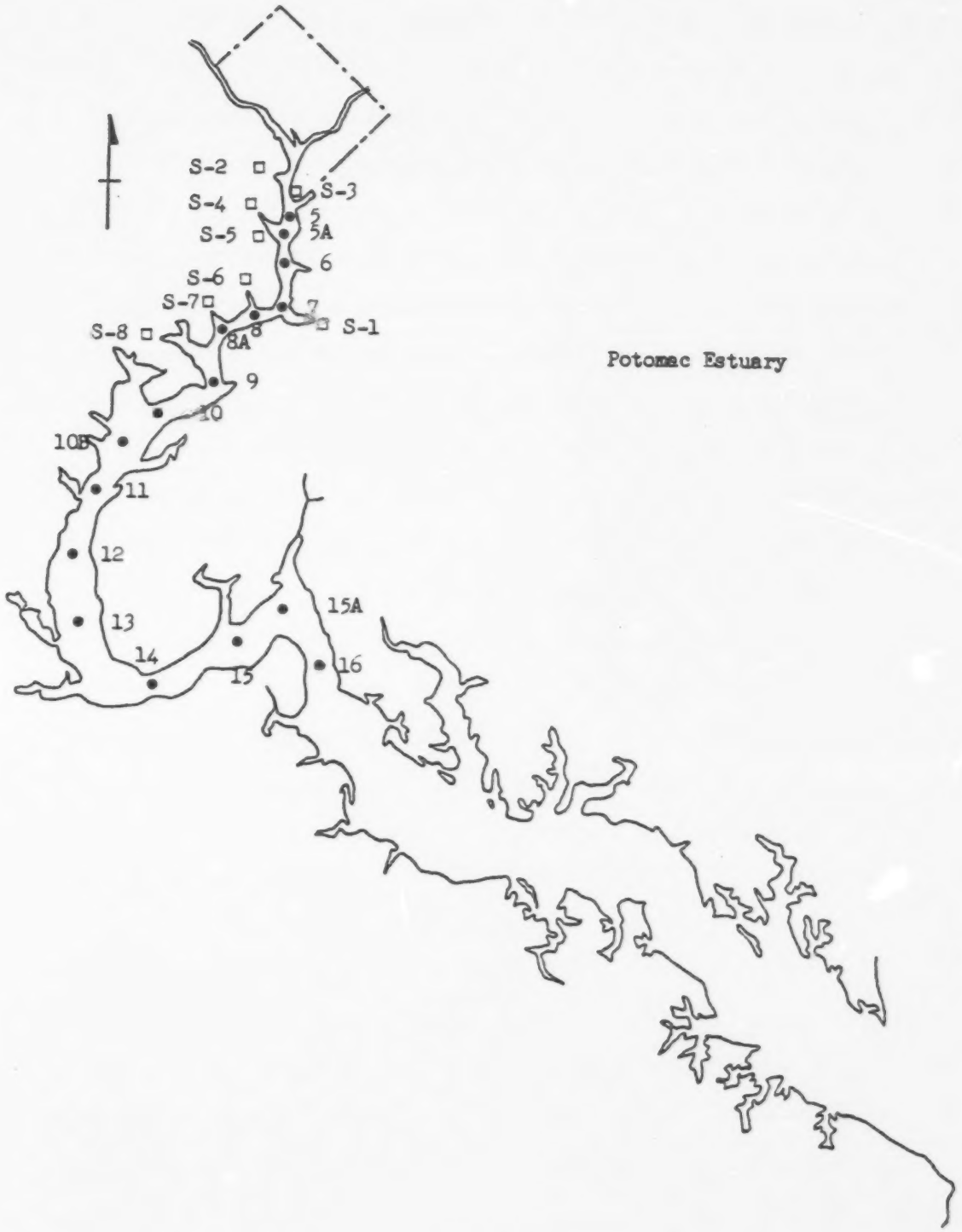


Table 1

Station Number	Station Name	RMI	Buoy Reference
P-8	Chain Bridge	0.0	
P-4	windy Run	1.9	
1	Key Bridge	3.4	
1-A	Memorial Bridge	4.9	
2	14th Street Bridge	5.9	
3	Hains Point	7.6	C "1"
4	Bellevue	10.0	FLR-23' Bell
5	Woodrow Wilson Bridge	12.1	
5-A	Rosier Bluff	13.6	C "87"
6	Broad Creek	15.2	N "86"
7	Ft. Washington	18.4	FL "77"
8	Dogue Creek	22.3	FL "67"
8-A	Gunston Cove	24.3	R "64"
9	Chapman Point	26.9	FL "59"
10	Indian Head	30.6	N "54"
10-B	Deep Point	34.0	
11	Possum Point	38.0	R "44"
12	Sandy Point	42.5	N "40"
13	Smith Point	45.8	N "30"
14	Maryland Point	52.4	G "21"
15	Nanjemoy Creek	58.6	N "10"
15-A	Mathias Point	62.8	C "3"
16	Rt. 301 Bridge	67.4	

Station Number	Treatment Plant Name
S-1	Piscataway STP
S-2	Arlington STP
S-3	Blue Plains STP
S-4	Alexandria STP
S-5	Westgate STP
S-6	Hunting Creek STP
S-7	Dogue Creek STP
S-8	Pohick Creek STP

and forced to draw upon reserve phosphate to meet their nutrient requirements. If phosphorus was depleted to a critical level in the estuary, measured concentrations of luxury phosphate would be expected to decrease and the activity of alkaline phosphatase would be expected to increase. Studies[3] have found that these changes are not immediate and a lag time occurs before the biological changes, related to phosphorus deficiency, are expressed.

Several species of algae, notably blue-green algae, have the ability to meet their nitrogen requirement by reducing free nitrogen (N_2) from the air and incorporating it into cellular organic compounds. Algae grown in an environment containing adequate fixed nitrogen (NH_4^+ or NO_3^-) do not fix N_2 without a preliminary starvation period during which the nitrogenase enzymes can develop.[4] The triple bonds of N_2 are extremely stable and breakage of these bonds involved in nitrogen reduction dictates that fixation requires considerable energy input. Cells capable of fixing nitrogen will use NH_4^+ or NO_3^- preferentially because less energy is required.[4,7]

The nitrogenase enzyme complex is comprised of two major protein components, Fe-protein and Mo-Fe-protein, each composed of several subunits.[12] Nitrogen is reduced by the enzyme complex to ammonia as electrons flow from a reducing agent to the Fe-protein, then to the Mo-Fe-protein and finally to nitrogen. The ammonia formed in these processes is subsequently employed in amino acids, which are the building blocks of protein. The nitrogen fixing activity of algae is often restricted to specialized cells termed heterocysts. These

are enlarged, clear (reduced pigmentation) cells, which apparently do not produce O_2 since oxygen is thought to deactivate nitrogenase.[10]

It has been found[13] that nitrogenase can reduce a variety of multiple bond substances in addition to molecular nitrogen. These include NO_2, N_3^-, RCN, RNC, and RCCH. Acetylene is reduced by this system to ethylene which is not further affected. Algae actively fixing nitrogen will produce ethylene when incubated with acetylene.

Bulk elemental analysis of the phytoplankton standing crop gave an indication of the carbon, nitrogen and phosphorus bound in algal cells. This information when ratioed to chlorophyll a gives a means of predicting algal C, N, and P from the more easily measured chlorophyll a concentration. To increase the comparability of these elemental analyses to cells of different sizes, the cell concentrations of C, N, and P were also reported on a dry weight basis. A problem with the comparability of elemental analysis is the varying amount of sheath material observed with different algal species.[4] This problem makes it difficult to establish a reliable relationship between elemental composition ratios measured and the nutrient status of the algae being studied.

II. Conclusions

A. The average composition of the phytoplankton present in the study area was (mg/ug):

Org C/ /chlor a = 0.028; PO_4/ /chlor a = 0.002; TKN-N/ /chlor a = 0.007

The predominate phytoplankton species present during the study period was the blue-green algae Oscillatoria spp.

B. No significant alkaline phosphatase activity was detected during this study and together with the average luxury phosphate of 0.45 mg PO_4/100 mg algae (dry) suggested that phosphorus was not limiting growth.

C. No significant nitrogen fixation was detected during the study period.

D. Ammonium uptake rate varied markedly with station location and a negative correlation, r = -.80 (n = 4), was determined for ammonia absorption rate vs $(NO_2 + NO_3)$-N concentration. The absorption rate increased from 0.0 ug NH_4^+-N/10 mg algae/hr. at a $(NO_2 + NO_3)$-N concentration of 0.352 mg/l at Chapman Point to 7.5 ug NH_4^+-N/10 mg algae/hr. when the nitrate + nitrite-nitrogen concentration became less than 0.04 mg/l at Possum Point. This indicated that the reach from Chapman Point to Possum Point was becoming nitrogen limited.

E. Approximately 50% of the algal TKN-N was refractory to the Technicon Autoanalyzer (phenolate/helix method) without preliminary manual digestion.

F. Elemental analysis data for phosphorus was obtained by Millipore filtration and by centrifugation. The results obtained were not significantly different.

III. Experimental

A. Chlorophyll a was determined on an untreated portion of the sample via a 90% acetone extraction of a Millipore filtrate from 100 ml of the sample.[5]

B. Sample preparation procedures (Figure 2) required the existence of a significant bloom (>50 ug/l chlorophyll a) so that errors due to "non-agal particulate material" would be minimized and so that sufficient algae could be concentrated to run the necessary tests. The sample preparation procedures involved:

1. Centrifuge algal sample in 50 ml aliquots (8) at 3K RPM for 5 minutes. The sample was stored at 4°C during this procedure.

2. Collect 10 ml of supernatant as a blank from each centrifuge tube in a 125 ml Erlenmyer flask stored on ice. Discard all but a few drops of the remaining liquid in the tubes.

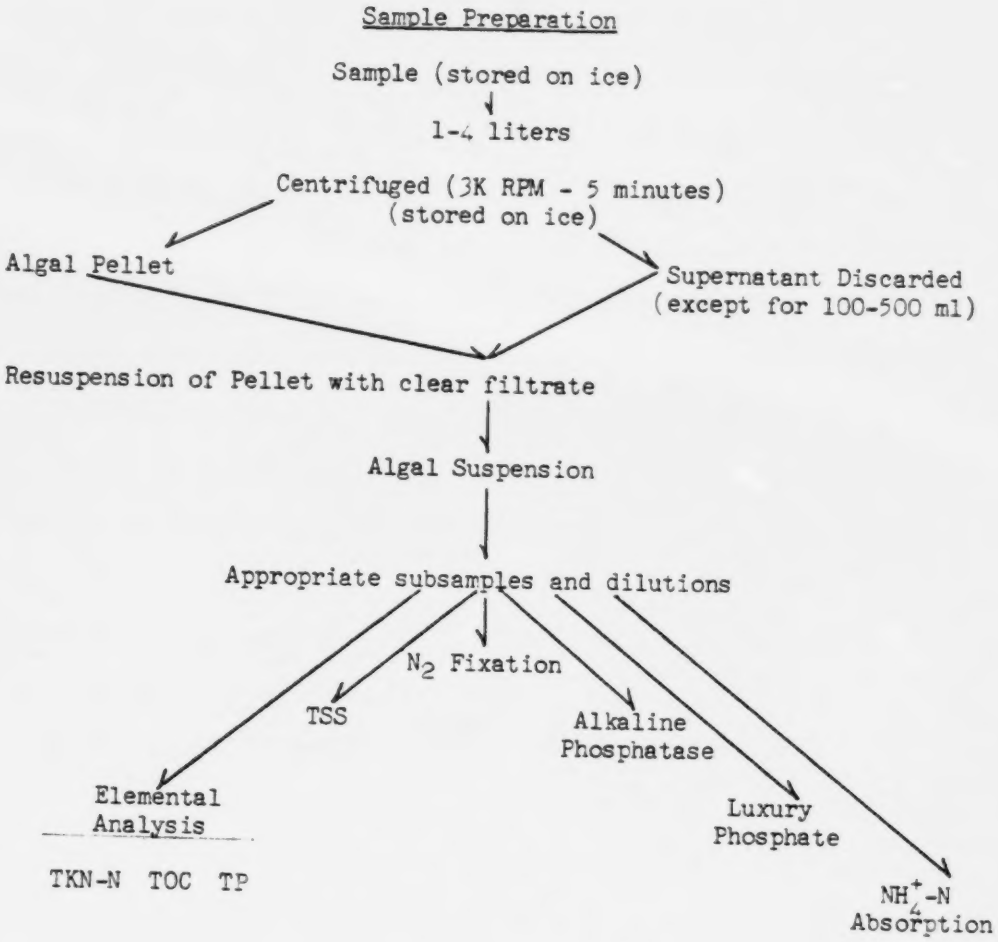
3. Resuspend pellets in ≥50 ml of river water blank (supernatant). The volume of the sample centrifuged and the volume to which the resultant algal pellet was diluted was recorded.

Microscopic examination revealed that no apparent morphological damage was suffered by the predominant phytoplankton species present.

C. Elemental Analyses

1. TKN-N (NH_3 plus organic nitrogen): 5 ml of algal suspension was diluted to 25 ml in a volumetric flask using Super Q - Milli Ro deionized water. A blank was run using

Figure 2


Sample Preparation
Sample (stored on ice)
1-4 liters
Centrifuged (3K RPM - 5 minutes)
(stored on ice)
Algal Pellet
Supernatant Discarded
(except for 100-500 ml)
Resuspension of Pellet with clear filtrate
Algal Suspension
Appropriate subsamples and dilutions
N_2 Fixation
TSS
Alkaline
Phosphatase
Elemental
Analysis
TKN-N TOC TP
Luxury
Phosphate
NH_4^+-N
Absorption

5 ml of supernatant river water diluted to 25 ml in Super Q - Milli Ro deionized water.

These samples were then manually digested: 10 ml aliquot of each was placed in reflux tubes and 8.0 ml of H_2SO_4/K_2SO_4 digestion solution was added. The tubes were placed over flame until boiling and reflux stopped. The contents of the tubes were washed with deionized water and brought to 50 ml using a graduated cylinder.

The resultant digests were analyzed using the Technicon Autoanalyzer phenolate method.[6]

2. TOC: 5 ml of algal suspension was diluted to 25 ml in a volumetric flask using Super Q deionized water. A blank was run using 5 ml of supernatant river water diluted to 25 ml in Super Q deionized water. The TC and IC were then determined on a Beckman 915 TOC analyzer.[6]

3. Total Phosphate: 25 ml of sample and blank were prepared as above by dilution of 5 ml of sample to 25 ml with deionized water. The sample and blank were placed in aluminum foil covered pyrex test tubes to which ammonium persulfate and sulfuric acid were added and autoclaved at 15 psi for 30 minutes. The digests were then analyzed for total phosphate by the Technicon automated ascorbic acid reduction method.[6]

D. Table 2

Growth Media[1] used in laboratory studies:

	Gorham's Complete Solution mg/l	Gorham's (Minus P) Solution mg/l	Gorham's (Minus N) Solution mg/l	Volume of Stock ml per liter	conc. stock
K_2HPO_4	39.0	0.0	39.0	1 ml	19.5g/500 ml
$NaNO_3$	496.0	496.0	0.0	10 ml	24.8g/500 ml
$MgSO_4 \cdot 2H_2O$	75.0	75.0	75.0	1 ml	37.5g/500 ml
$CaCl_2 \cdot 2H_2O$	36.0	36.0	36.0	1 ml	18.0g/500 ml
$Na_2SiO_3 \cdot 9H_2O$	58.0	58.0	58.0	10 ml	2.9g/500 ml
Na_2CO_3	20.0	20.0	20.0	1 ml	10.0g/500 ml
Ferric Citrate	6.0	6.0	6.0	10 ml	0.3g/500 ml
Citric Acid	6.0	6.0	6.0	1 ml	3.0g/500 ml
(Na_2)E.D.T.A.	1.0	1.0	1.0	1 ml	1.0g/500 ml

E. Luxury Phosphate[2]

1. Spin down two sets of 5 ml aliquots of algal suspension at 3K RPM for 5 minutes and discard supernatant.
2. Lightly wash pellet with 10 ml of Gorham's (P-minus) solution adjusted to pH 7 with acetic acid.
3. Pour off liquid and wash cells with Gorham's (P-minus) pH 7 solution into an Erlenmyer flask to a total volume of 40 ml.
4. Cover with aluminum foil and place one flask into boiling water for 60 minutes.
5. The other set is immediately centrifuged and the supernatant analyzed for PO_4.
6. After one hour repeat step #5 for the first extracted set.

7. Calculate the net (by difference) extracted PO_4/ 100 mg algae (dry weight).

Definition: Extracted algae that give less than 0.03 mg PO_4/ 100 mg algae (dry weight) are considered to be phosphorus limited.

F. Alkaline Phosphatase Activity[2]

1. Centrifuge 5 ml of algal suspension and discard supernatant.
2. Wash pellet with 10 ml of Gorham's (P-minus) adjusted to pH 9.0 with acetic acid.
3. Wash cells into Erlenmyer flask with 32 ml of Gorham's (P-minus) pH 9.0 solution.
4. Add 4 ml of 1M TRIS solution which is also 0.01 M $MgCl_2$ and adjust pH to 8.5 with acetic acid.
5. Add 4 ml of p-nitrophenyl phosphate solution (30 mg/100 ml).
6. Incubate glass stoppered flask with mixing for 15 to 20 minutes at 35-37°C.
7. Stop when color is within standard curve by adding 0.5 ml of orthophosphate (20 mg PO_4/ml) stock solution.
8. Filter material through .45 u Millipore membrane filter and analyze liquid.
9. Read absorbance at 395 nm in 2 cm cells with 2.0 nm slit.
10. Run standard curve of nitrophenol, (color is pH dependent) with:

32 ml Gorham's (P-minus) adjusted to pH 9.

4 ml of the Tris Buffer.

4 ml of standard solution.

11. Standard curve concentrations (after reagent addition): 0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 $\times 10^{-5}$M p-nitrophenol.

a. Preparation of standard solutions:

(1) Prepare a stock of p-nitrophenol of 1.3911g/l (10^{-2}M).

(2) 20 ml of this solution was diluted to 200 ml with deionized water to generate a working stock.

(3) 5; 10; 15; 20; 25; and 30 ml of the working solution is diluted to 100 ml with deionized water to generate: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 $\times 10^{-4}$M solutions.

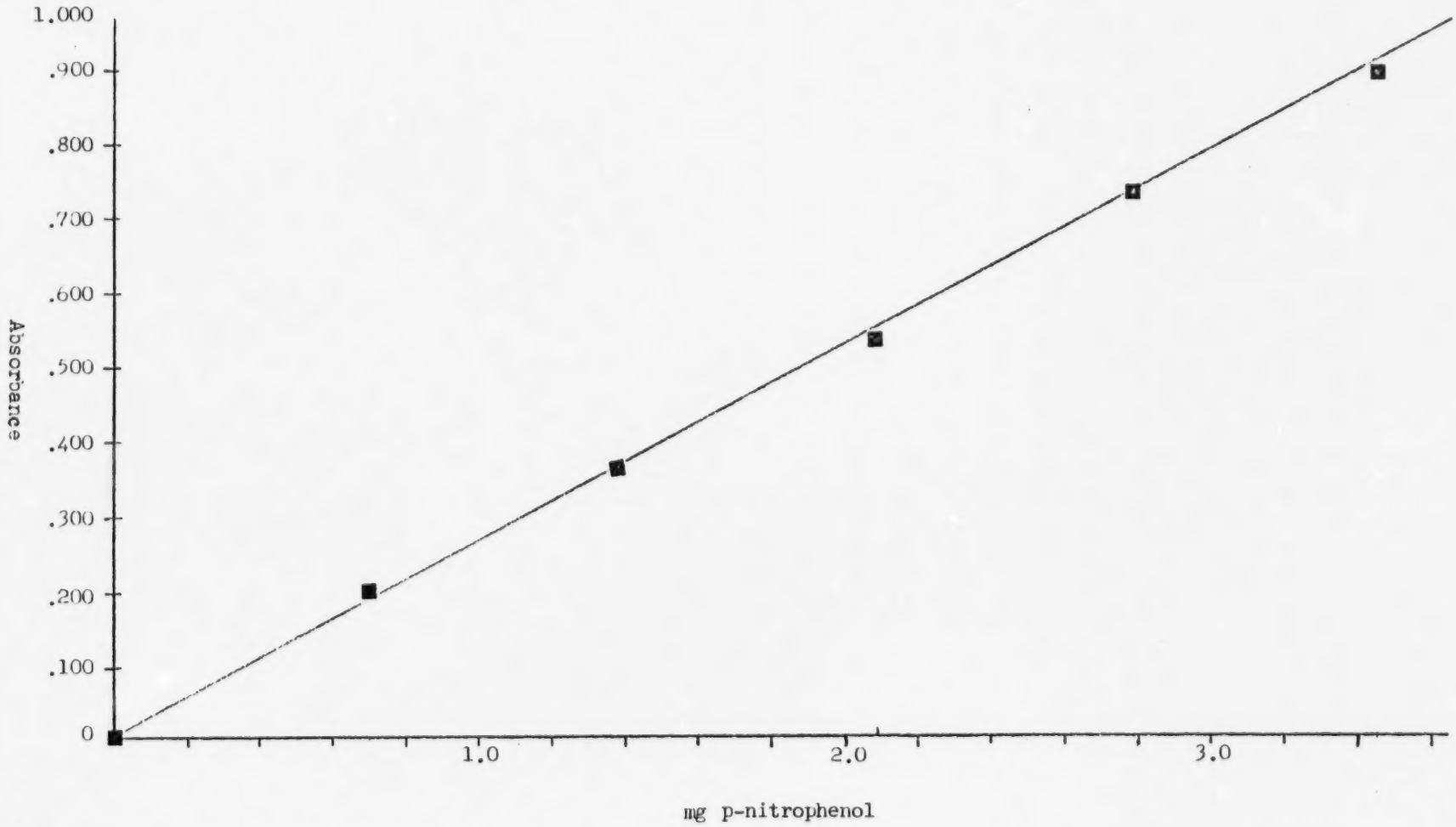
(4) When 4 ml of these solutions is diluted to 40 ml total with reagent, the standard curve at the 10^{-5}M level is generated.

b. Characteristic Calibration Curve (Figure 3)

Concentration	Absorbane	mg p-nitrophenol
0.0	0.000	0.00
0.5 x 10^{-5}M	0.193	0.70
1.0 x 10^{-5}M	0.362	1.39
1.5 x 10^{-5}M	0.536	2.08
2.0 x 10^{-5}M	0.733	2.78
2.5 x 10^{-5}M	0.902	3.45
3.0 x 10^{-5}M	1.076	4.17

Figure 3

STANDARD CURVE FOR ALKAKINE PHOSPHATASE ENZYME ACTIVITY



12. Determine mu moles of nitrophenol liberated/hr. per milligram of algae (dry weight).

Definition:

a. 1 unit of enzyme activity is equivalent to 1.0 mu mole of nitrophenol per hour per mg dry weight.

b. $\sim$ 1000 enzyme units/mg algae/hour represent algae considered phosphorus limited.

c. This test is generally a confirming test since changes in enzyme activity per changes in nutrient level are slow to occur.

A check standard of bacterial alkaline phosphatase (12 units/mg from the Worthington Biochemical Corporation) was run as a positive control check with each batch of samples analyzed.

G. Ammonia Absorption Rate[7]

1. Centrifuge 2 sets of 5 ml aliquots of algal suspension at 3K RPM for 5 minutes. Discard the supernatant.

2. Pre-wash pellets with 10 ml of Gorham's N-minus, adjusted to pH 8.0 with acetic acid and discard liquid.

3. Wash pellets into a flask with 30 ml of Gorham's N-minus adjusted to pH 8.0.

4. Spike both sets with 0.5 mg NH_4Cl-N/l.

5. Centrifuge the first set immediately and analyze supernatant for NH_4^+-N.

6. Incubate the other flask in the dark at 68°C with occasional mixing for one hour.

7. Centrifuge and assay supernatant for NH_4^+-N.

Threshold Limit: Nitrogen-starved algal cells were found to assimilate NH_4^+-N 4 to 5 times more rapidly than normal cells under optimum nitrogen conditions. The limit cited is that algae are considered nitrogen limited if they absorb more than 15 ug NH_4^+-N/10 mg dry algae per hour[7]. This threshold rate, however, was observed to vary from species to species. The comparison of NH_4^+-N assimilation rates measured for algae from different locations in the Potomac River study area, associated with different in situ nitrogen concentrations was thought to be more meaningful. A drastic rate of increase (~4 or 5 times) from one location to another was taken to indicate changes in the availability of nitrogen for assimilation purposes and suggested that nitrogen was limiting growth.

H. N_2 Fixation[8]

1. Sample preparation:

   a. Concentrate 2 liters of sample (770906-16, 17, 19) for algae as described previously and bring to 25 ml total volume with river water supernatant. (Blank)

   b. Add 10 ml of each concentrate to two 40 ml septum vials.

2. Seal the vials with an injectionable septum (air tight pharmaceutical type).

3. Inject 1.5 ml of acetylene (C_2H_2) into each vial using a 5 ml disposable syringe.

4. Immediately inject 0.2 ml of 5N H_2SO_4 into one set to act as a control blank.

5. Shake all flasks and vent by pricking with a hypodermic needle.

6. Incubate in a water bath in direct sunlight for 1 1/2 hours at 29°C (~ambient surface water temperature).

7. The reduction reaction was stopped by the injection of 0.2 ml of 5N H_2SO_4. The samples were stored at 4°C until gas chromatographic analysis.

8. The G.C. and experimental conditions were as follows:

   a. Column temperature: 50°C

   b. Flow 25 ml/minute of Helium

   c. Column: porapack N, 80-100 mesh, 6 ft. with 0.2 mm I.D.

   d. Retention time:

      Ethylene: 1.75 minute

      Acetylene: 3.55 minute

   e. Room temperature: 24°C; 30.13" Hg barometric pressure.

9. Chlorophyll a concentration was determined as described previously, and using the measured TKN-N/chlorophyll a ratio (0.007) the mg of algal TKN-N was determined and the results were reported as ng acetylene reduction per mg algal TKN-N.

10. The volume of the vials (60.0 ml) was determined using the weight of water at room temperature.

11. The procedure for diluting the stock ethylene was crude but the reduction test was run more as a qualitative assay to detect significant nitrogen fixation rather than a strictly quantitative rate determination. The dilution and spikes were as follows:

a. Stock ethylene preparation (m.w. 28.04 gm/mole)

(1) Ethylene was assumed an ideal gas or PV = nRT.

$R = 0.082$ l atm K^{-1} mol^{-1}

$T = 24^{\circ}C$ or 297.14 K

$P = 30.13$" Hg x 2.54 cm/in x $\frac{1 \text{ ATM}}{76 \text{ cm}} = 1.0070$ ATM

$V = 60$ ml bottle or 0.060 l

$n = \frac{PV}{RT} = \frac{(1.007)\ (0.060)}{(0.082)\ \ (297)} = 0.00248$ moles of gas in stock

(2) Inject 0.5 ml directly into 60 ml gas tight vial:

$\frac{.00248 \text{ moles}}{60 \text{ ml}}$ x .5 ml = .000021 moles or .578 mg

b. Dilute 5/60 by volume using a gas tight syringe and gas tight bottle:

.00248 moles x $\frac{5}{60}$ = .000207 moles in dilution

(1) Inject 0.5 ml directly:

$\frac{.000207}{60 \text{ ml}}$ moles x 0.5 ml = .00000173 mole or .049 mg

(2) Inject 1.0 ml directly:

$\frac{.000207}{60 \text{ ml}}$ moles x 1.0 ml = .0000034 mole or .095 mg

c. Dilute 1/60 by volume using a gas tight syringe and gas tight bottle:

.00248 moles x $\frac{1}{60 \text{ ml}}$ = .0000413 moles in dilution

Inject 0.5 ml directly:

$\frac{.0000413}{60}$ x 0.5 ml = .00000034 mole or .0095 mg

The area of the G.C. peaks for these standards was used to determine the concentration in the unknown samples.

IV. Discussion of Results

The elemental analyses and special bioassay results are compiled in Table 3. The location and chlorophyll a distribution of the stations sampled for this study are given in Figures 4-7. It should be emphasized that these results are based on the overall phytoplankton standing crop. The alkaline phosphatase activity (>1000 enzyme units/mg algae/hour)[2] indicative of phosphorus starved algal cells was not encountered in any of the study samples. The average luxury phosphate measured, 0.45 mg PO_4/100 mg algae (dry), was in excess of the established threshold level for phosphorus limitation of 0.03 mg PO_4/100 mg algae (dry).[2] Little difference was observed in luxury phosphate measured at the upstream and downstream stations. The inorganic phosphate concentration increased in the bloom area with an average of 0.214 mg/l PO_4 measured over the study stations. The alkaline phosphatase, luxury phosphate, and ambient inorganic phosphorus data indicated that adequate phosphorus was present for maximum growth during the study period.

The inorganic nitrogen source for algal growth was limited to $(NO_2 + NO_3)$-N in the bloom area, Table 4. This was a result of the rapid nitrification of the ammonia entering the river upstream of the study area.[9] The distribution of measured ammonium uptake rates relative to $(NO_2 + NO_3)$-N measured in the Potomac are also included in Table 4. Though this data is sparse, a significant increase in uptake rate occurred with $(NO_2 + NO_3)$-N depletion on the August 29 analysis between Chapman Point (0.0 ug NH_4^+/10 mg algae/hour) and Possum Point (7.5 ug NH_4^+/10 mg algae/hour). This data (n = 4) was used to generate the correlation coefficient of -0.8. The increased

Table 3

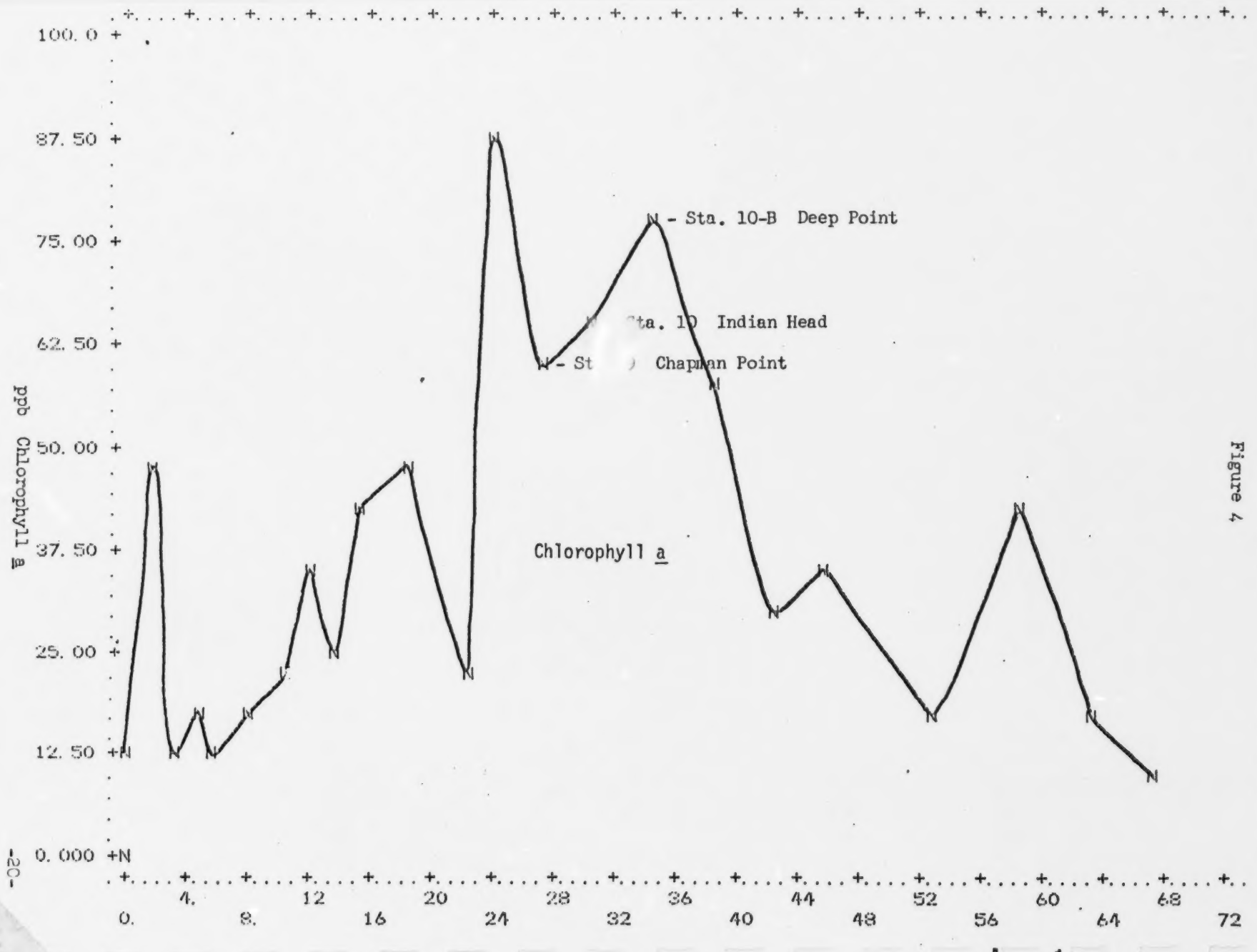
SUMMARY OF ASSAY/ANALYSIS RESULTS

Location	Date	Sta.	mg/ug $\frac{C_{org}}{Chl.\ a}$	mg/mg $\frac{C_{org}}{Tss}$	mg/ug $\frac{PO_4}{Chl.\ a}$	mg/mg $\frac{PO_4}{TSS}$	Sample TP	Sample Pi	mg/ug $\frac{TKN\text{-}N}{Chl.\ a}$	mg/mg $\frac{TKN\text{-}N}{TSS}$	1 Luxury Phosphate	2 Alkaline Phosphatase	3 NH_4	ug/l Chl. a
Chapman Pt.	8-1-77	9	.036	.152	.003	.014	.503	.161	.013	.055	.37	ND	0.3	60.0
Indian Head	8-1-77	10	.028	.213	.002	.017	.472	.157	.008	.060	-	-	-	66.0
Deep Pt.	8-1-77	10-B	.029	.211	.002	.015	.469	.157	.009	.065	-	-	-	76.5
Gunston Cove	8-22-77	8-A	.037	.238	.003	.018	.764	..227	.008	.052	.25	ND	0.3	306
Chapman Pt.	8-22-77	9	.026	.283	.002	.018	.751	.238	.006	.060	.38	ND	0.2	264
Indian Head	8-22-77	10	.037	.157	.003	.012	.736	.259	.008	.035	.15	ND	0.3	283.5
Chapman Pt.	8-29-77	9	.013	.162	.001	.015	.799	.176	.003	.038	.51	ND	0.0	261
Indian Head	8-29-77	10	.012	.213	.001	.022	.759	.205	.003	.058	.67	ND	0.4	300
Deep Pt.	8-29-77	10-B	.027	.376	.001	.016	.850	.275	.004	.052	.53	ND	4.2	294
Possum Pt.	8-29-77	11	.034	.433	.002	.020	.846	.282	.005	.050	.73	ND	7.5	199.5
	Average		.028	.244	.002	.017	.694	.214	.007	.053	.45	<57 E.U.		

1. Luxury Phosphate: mg PO_4/100 mg Algae (dry)

2. Alkaline Phosphatase: 1 E.U. = 1.0 mu Moles Nitrophenol/mg algae/hour

3. NH_4^+-N Absorption: ug NH_4^+-N/10 mg Algae/hour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87. 50
75. 00
62. 50
50. 00
37. 50
25. 00
12. 50
0. 000
ppb Chlorophyll a
-20-
N - Sta. 10-B Deep Point
Sta. 10 Indian Head
St Chapman Point
Chlorophyll a
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4.
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16
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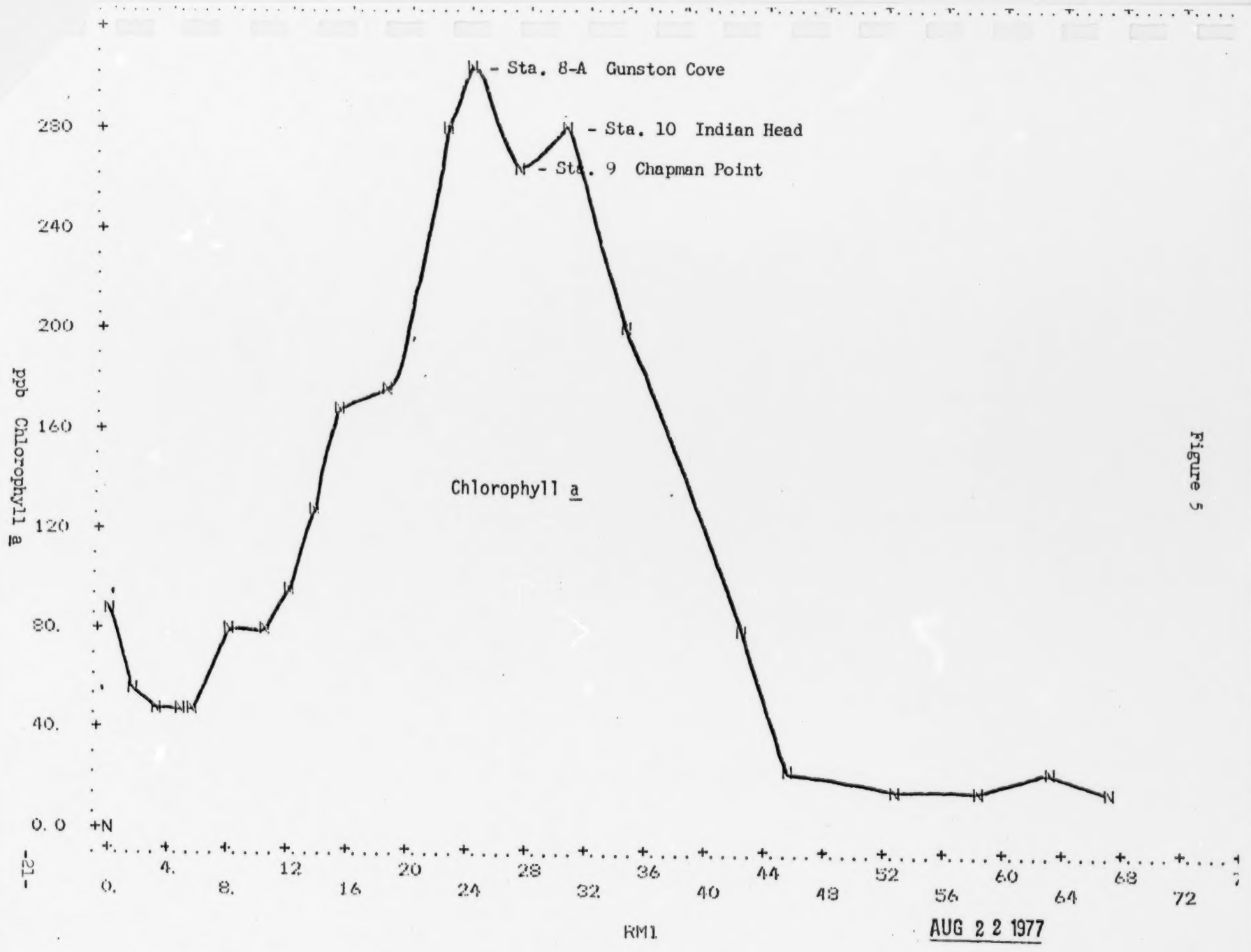
72

Figure 4


N - Sta. 8-A Gunston Cove
N - Sta. 10 Indian Head
N - Sta. 9 Chapman Point
Chlorophyll a
ppb Chlorophyll a
280
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200
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120
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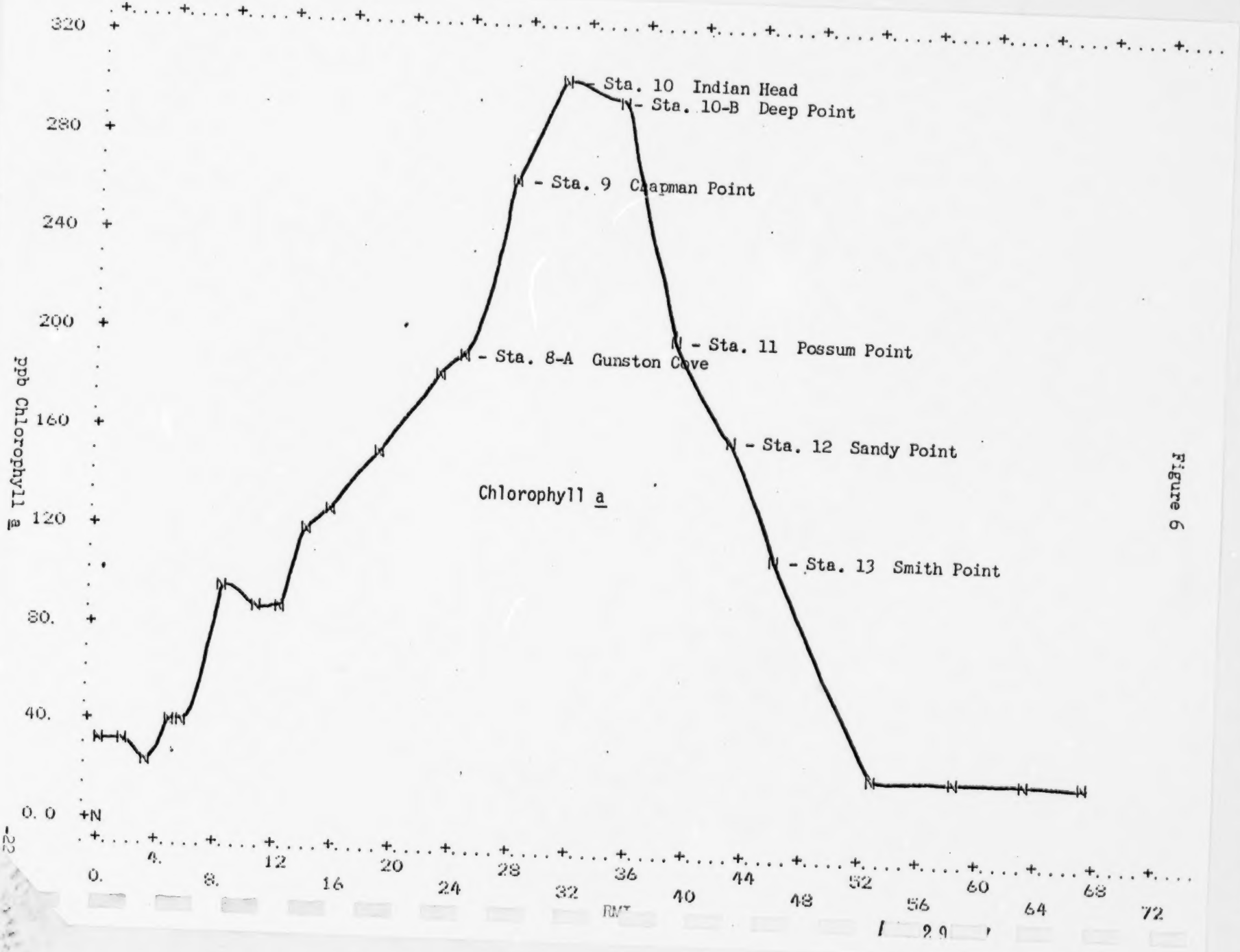
Figure 5


320
280
240
200
160
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80.
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0.0
ppb Chlorophyll a
Sta. 10 Indian Head
Sta. 10-B Deep Point
Sta. 9 Chapman Point
Sta. 8-A Gunston Cove
Sta. 11 Possum Point
Sta. 12 Sandy Point
Sta. 13 Smith Point
Chlorophyll a
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RMT

Figure 6

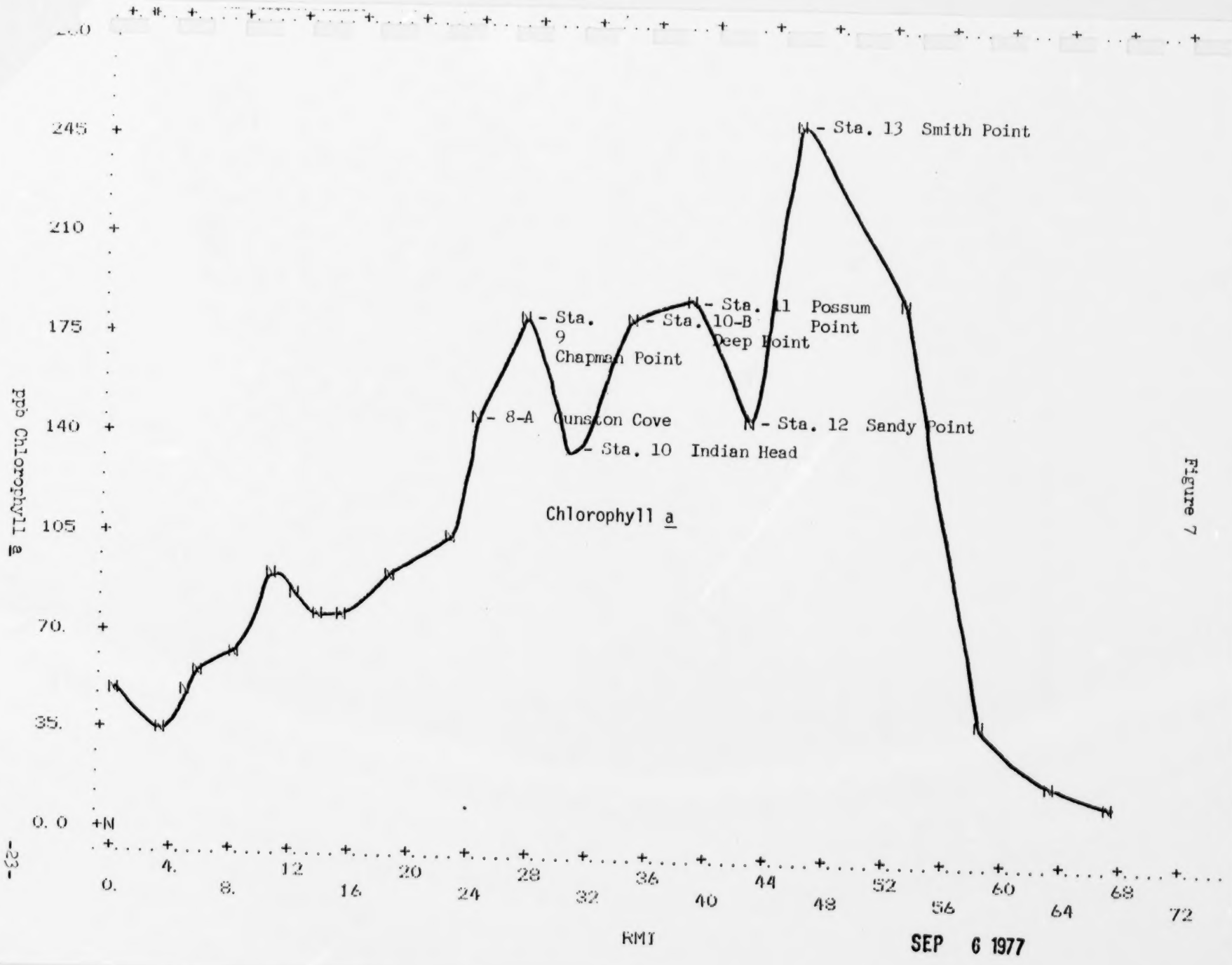
245
210
175
140
105
70.
35.
0. 0
ppb Chlorophyll a
- Sta. 13 Smith Point
- Sta. 9 Chapman Point
- Sta. 10-B Deep Point
- Sta. 11 Possum Point
- 8-A Gunston Cove
- Sta. 12 Sandy Point
- Sta. 10 Indian Head
Chlorophyll a
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Figure 7

Table 4

Ammonium Uptake Rates/Nitrogen Distribution

Location	Date	Sta.	ug NH_4^+-N/10 mg Algae/hour	mg NH_4^+-N/ug Chl. a/hour
Chapman Pt.	8-1-77	9	0.3	0.4×10^{-5}
Gunston Cove	8-22-77	8-A	0.3	0.5×10^{-5}
Chapman Pt.	8-22-77	9	0.2	0.2×10^{-5}
Indian Head	8-22-77	10	0.3	0.5×10^{-5}
Chapman Pt.	8-29-77	9	0.0	0.0
Indian Head	8-29-77	10	0.4	8.0×10^{-5}
Deep Pt.	8-29-77	10-B	4.2	12.2×10^{-5}
Possum Pt.	8-29-77	11	7.5	23.7×10^{-5}

Location	Date Sta.	8-1-77 *$NH_4^+\uparrow$	8-1-77 $(NO_2 + NO_3)$-N	8-22-77 $NH_4^+\uparrow$	8-22-77 $(NO_2 + NO_3)$-N	8-29-77 $NH_4^+\uparrow$	8-29-77 $(NO_2 + NO_3)$-N
Gunston Cove	8-A		.928	0.3	.181		
Chapman Pt.	9		.710	0.2	.130	0.0	.352
Indian Head	10	0.3	.495	0.3	.089	0.4	.110
Deep Pt.	10-B		.378		ND	4.2	ND
Possum Pt.	11		.122		ND	7.5	ND
Sandy Pt.	12		**ND		.126		ND
Smith Pt.	13		ND		.317		ND

Note: The NH_4^+-N concentration was less than 0.02 mg/l over these dates and stations except for Sta. 13 on 8-22-77 which had an NH_4^+-N concentration of 0.052 mg/l.

*$NH_4^+\uparrow$ = ug NH_4^+-N/10 mg Algae/hour

**ND = not detectable = <0.04 mg $(NO_2 + NO_3)$-N/l

rate of ammonium absorption ($\geq$7.5x) corresponded to a decrease in inorganic nitrogen from 0.352 mg/l $(NO_2 + NO_3)$-N at Chapman Point to less than 0.04 mg $(NO_2 + NO_3)$-N/l at Possum Point. The rate of NH_4^+-N absorption by algae and aquatic weeds in the dark has been shown to be 4-5 times greater for plants which are N-limited as compared to plants with sufficient available nitrogen.[7] This indicated that this reach of the Potomac, Chapman Point to Possum Point, was becoming nitrogen limited.

As the $(NO_2 + NO_3)$-N source was depleted it was of interest whether nitrogen fixation might be occurring. Nitrogen fixation had been observed in a marine species of Oscillatoria,[10] but fresh water species are considered non-N_2 fixers.[11] In general algae may utilize N_2; NH_3; or $(NO_2 + NO_3)$-N as their nitrogen source if the proper enzyme systems are present and activated. The use of N_2 and $(NO_2 + NO_3)$-N require additional reactions and energy. Algae capable of utilizing these sources of nitrogen preferentially select ammonia, since it involves the most efficient source of cellular nitrogen.[4,7] The nitrogen fixation data is compiled in Table 5 and indicates that no significant acetylene reduction ($\leq$.073 n moles C_2H_4/mg N/hour) was measured although ambient inorganic nitrogen became non-detectable between Deep Point and Sandy Point on September 6 when the acetylene fixation procedure was carried out. Values of 126-230 n moles C_2H_4/mg N/hour have been reported as indicative of high efficiencies of acetylene reduction,[11] and rates of 30-60 n moles C_2H_4/mg N/hour are considered significant.[8]

As a check on the laboratory procedures involved in centrifugation

Table 5

N_2 FIXATION/ACETYLENE REDUCTION

Ethylene Measurements

Location	Date	Sta.	Vol. Injected cc	Ethylene Area	Net Area (Blk)	Ethylene (mg)	Acetylene Area
Acetylene Srandard			0.2				
Air Blank			0.5	637 (ND Level)			10, 129, 733
Deep Pt.	9-6-77	10-B	5.0	1541	0	0	10, 894, 579
		10-B Blk	5.0	2158			12, 074, 981
Possum Pt.	9-6-77	11	5.0	2103	289	ND	11, 794, 779
		11 Blk	5.0	1817			11, 672, 681
Sandy Pt.	9-6-77	12	5.0	2225	302	ND	10, 765, 493
		12 Blk	5.0	1923			11, 584, 374
Smith Pt.	9-6-77	13	5.0	2950	709	$8.7\ 10^{-6}$ mg	11, 032, 906
		13 Blk	5.0	2241			10, 681, 130
Standards:							
Ethylene 5/60			0.5	4,150,447		.049 mg	
Ethylene 5/60				3.781.067		.049 mg	
				$\bar{x}$ 3,965,757			

Agal TKN-Nitrogen (Ethylene Experiment)

Location	Date	Sta.	Volume of Sample Concentrated	Final Volume of Suspension	Volume Incubated	TKN-N/ Chl. a	Chl. a Conc. in Orginal Sample ug/l	mg Algal TKN-N	Ambient Inorganic Nitrogen (mg/l) $(NO_2 + NO_3)$-N	NH_3-N
Indian Head	9-6-77	10	-	-	-	-		-	.725	ND (< .04)
Deep Pt.	9-6-77	10-B	2 liters	25 ml	10 ml	.007	180	1.008	.172	ND

Table 5 (Continued)

Agal TKN-Nitrogen (Ethylene Experiment) (Continued)

Location	Date	Sta.	Volume of Sample Concentrated	Final Volume of Suspension	Volume Incubated	TKN-N/ Chl. a	Chl. a Conc. in Orginal Sample ug/l	mg Algal TKN-N	Ambient Inorganic Nitrogen (mg/l) $(NO_2 + NO_3)$-N	NH_3-N
Possum Pt.	9-6-77	11	2 liters	25 ml	10 ml	.007	186	1.042	ND	ND (< .04)
Sandy Pt.	9-6-77	12	2 liters	25 ml	10 ml	.007	145.5	.815	ND	ND
Smith Pt.	9-6-77	13	2 liters	25 ml	10 ml	.007	253.5	1.420	ND	.045

Ethylene/TKN

Location	Date	Sta.	n Moles C_2H_4/30 minute/mg N
Deep Pt.	9-6-77	10-B	0
Possum Pt.	9-6-77	11	0
Sandy Pt.	9-6-77	12	0
Smith Pt.	9-6-77	13	0.073

and sample concentration, dilution, etc., the elemental analysis results were compared to a second method (filter method). The results are compiled in Table 6. The columns designated "total" represent analytical results (C, N, and P) on the unaltered samples. The column labeled "filtered" represents the elemental analyses of the filtrate after filtration through 0.45 u Millipore filters. The algae were held on the filter and the differences of filtered and unfiltered results were taken as the algal material. A paired t-test of the phosphorus results revealed that there was no significant difference at the 95% confidence level and 9 degrees of freedom between the results of the two methods with t = 1.195. The nitrogen data (Table 6) was consistently lower for the filtered experiments. The TKN-N for the filtered data did not incorporate the preliminary manual digestion used in the centrifuge procedure. The results suggest that 50% of the algal nitrogen was refractory to the TKN-N Technicon Autoanalyzer without preliminary manual digestion. A paired t-test of filtered TKN-N data (corrected for recovery) and centrifuged data established that there was no significant difference at the 95% confidence level and 9 degrees of freedom with t = 0.958. The good comparison between these experimental approaches suggest that the analytical procedures were accurate and precise. The basic assumption inherent in both was that the primary suspended material was algae. This assumption was not tested but algae assays and analyses were limited to the peak-bloom area where the assumption would be most reasonable.

Table 6

FILTERED vs CENTRIFUGED METHODS

Location	Date	Sta.	Total TP	Total Pi	Filtered TP	Filtered Pi	Chl. a	org PO_4/Chl. a Filter	org PO_4/Chl. a Centrifuge
			mg PO_4/l				ug/l		
Chapman Pt.	8-1-77	9	.503	.161	.204	.102	60.0	.004	.003
Indian Head	8-1-77	10	.472	.157	.212	.114	66.0	.003	.002
Deep Pt.	8-1-77	10-B	.469	.157	.210	.120	76.5	.003	.002
Gunston Cove	8-22-77	8-A	.764	.162	.240	.227	306	.002	.003
Chapman Pt.	8-22-77	9	.751	.238	.243	.170	264	.002	.002
Indian Head	8-22-77	10	.736	.259	.270	.207	283.5	.002	.003
Chapman Pt.	8-29-77	9	.799	.176	.256	.122	261.0	.002	.001
Indian Head	8-29-77	10	.759	.205	.250	.136	300.0	.001	.001
Deep Pt.	8-29-77	10-B	.850	.275	.279	.290	294	.002	.001
Possum Pt.	8-29-77	11	.846	.282	.366	.310	199.5	.002	.002
							$\bar{x}$ =	.002	.002
							v = 9		
							t = 1.152		
							r = .50		

Location	Date	Sta.	Total TKN	Total NH_3	Filtered TKN	Filtered NH_3	Chl. a	N org/Chl. a Filter	N org/Chl. a Centrifuge	Filter x2
			mg N/l				ug/l			
Chapman Pt.	8-1-77	9	.685	ND	.338	ND	60.0	.006	.008	.012
Indian Head	8-1-77	10	.651	ND	.313	ND	66.0	.005	.013	.010
Deep Pt.	8-1-77	10-B	.600	ND	.288	ND	76.5	.004	.009	.008
Gunston Cove	8-22-77	8-A	1.439	ND	.344	ND	306	.004	.008	.008
Chapman Pt.	8-22-77	9	1.254	ND	.362	ND	264	.003	.006	.006
Indian Head	8-22-77	10	1.227	ND	.353	ND	283.5	.003	.008	.006
Chapman Pt.	8-29-77	9	1.378	ND	.526	ND	261.0	.003	.003	.006
Indian Head	8-29-77	10	1.328	ND	.426	ND	300.0	.003	.003	.006
Deep Pt.	8-29-77	10-B	1.111	ND	.342	ND	294	.003	.004	.006
Possum Pt.	8-29-77	11	.885	ND	.334	ND	199.5	.003	.005	.006
							$\bar{x}$ =	.004	.007	.007
							v = 9.00			
							t = .958			
							r = .67			

V. Recommendations

A. It is recommended that future work with algal bioassays be split into two areas of concern. Algae from the peak bloom area (highest chlorophyll a concentration) should be employed in the elemental analysis work. This will ensure adequate phytoplankton necessary for the required analyses. Limiting nutrient analyses should be stressed in areas downstream from the peak bloom, where algae are encountering less productive conditions.

B. It is recommended that future N_2-fixation work involve concentration and incubation of phytoplankton in situ. In addition to providing the natural setting for incubation, larger quantities of algae should be obtained to insure that the TKN-N determinations are in the optimal range of the test. The practice of reporting acetylene reduction in terms of total Kjeldahl nitrogen limits the test to some degree by the lack of sensitivity of the TKN-N analysis relative to the gas chromatographic determination of ethylene.

VI. References

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